SLAC we have collected femtosecond pulsed coherent X-ray scattering from many molecular systems. It has been found that sufficiently brief X-ray pulses terminate before radiation damage commences, opening up many opportunities for new experiments in time- resolved imaging with atomic spatial resolution at room temperature, in condensed matter physics, materials science and biology. I will review some of this work, undertaken in collaboration with many others, including pump-probe experiments on the large molecular complexes involved in photosynthesis, and on a drug target molecule for sleeping sickness. A new approach to disentangling orientational disorder will also be demonstrated, aimed at reconstructing the image of one molecule, using the scattering from many in random orientations in solution, without modeling, based on angular correlation functions. A new single-particle injection scheme will be described to synchronize X-ray pulse emission with particle injection. Prospects for the formation of ''molecular movies'' which track chemical reactions will be outlined. I'll also describe the new approaches to the phase problem which these experiments suggest. A review of all this work can be found in Rev Mod Phys. 75, 102601 (2012)

193-Symp
The Next 100 Years of Crystallography: How the Heck should I Know?
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''There was a door to which I found no key: There was the veil through which I might not see.''於 - Omar Khayyam
''Take therefore no thought for the morrow: for the morrow shall take thought for the things of itself. Sufficient unto the day is the evil thereof.'' [Matthew 6:34]
''Tomorrow is an old deceiver, and his cheat never grows stale.'' 於- Samuel Johnson
''I know of no way of judging the future but by the past.'' 於- Patrick Henry
''I never think of the future. It comes soon enough.''於 - Albert Einstein
''When men speak of the future, the gods laugh.'' 於- Chinese Proverb
''L'avenir est comme le reste: il n'est plus ce qu'il était'' - Paul Valery
''It's tough to make predictions, especially about the future.'' 於- Yogi Berra

Symposium: Liquid Protein Assemblies in Spatial Organization and Ultrasensitive Signaling in Cells

194-Symp
Phase Separation of Disordered Protein in the Formation of Membrane-Less Organelles
Timothy J. Nott[1], Patrick Farber[2,3], Evangelia Petsalakis[1], Dylan Jervis[4], Andrew J. Baldwin[5], **Julie D. Forman-Kay**[2,3].
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Intrinsically disordered proteins and regions (IDPs/IDRs), which do not have stable secondary and tertiary structure, are capable of adopting different structural states. Many IDPs/IDRs populate conformationally heterogeneous monomeric states or engage in discrete interactions with other proteins, leading to folding upon binding or retaining significant disorder in the bound state. Others are involved in large-scale association having different degrees of order, from more defined fibers, to variably networked gels and to disordered liquid demixed states or droplets. These latter have been suggested to provide the physical basis for cellular membrane-less organelles such as the nucleolus.
We have studied the N-terminal disordered region of Ddx4, an RNA DEAD-box helicase that is essential for formation of a class of membrane-less organelles termed nuage or germ granules functioning in spermatogenesis. When expressed in HeLa cells, the protein forms spherical, micron-sized, liquid-like cellular organelles. In vitro, it phase separates to form droplets with similar morphological and dynamic properties to the organelles observed in cells. Phase separation is sensitive to salt, pointing to the importance of electrostatic interactions. The sequence features of the disordered N-terminus of Ddx4 that underlie phase separation include clustering of charged residues into blocks of net positive and negative charge, with over-representation of FG/GF pairs and RG/GR pairs within the positive blocks. Perturbations of these properties disrupt phase separation, pointing to multi-valent cation-pi interactions playing an important role. The transient sampling of multi-valent interactions in self-association of Ddx4 extends previous observations of dynamic multi-valent interactions in discrete complexes of IDPs/IDRs, such as for Sic1:Cdc4 binding. The insights obtained from these and ongoing biophysical studies of Ddx4 will be valuable for developing a general understanding of the biogenesis and disassembly of membrane-less cellular organelles.

195-Symp
The Liquid State of (Elastomeric) Proteins
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Self-assembled elastomeric proteins make up an important and unusual class of structural proteins endowing biological tissues as diverse as spider silks and lung alveoli with extensibility and elastic recoil. In humans, elastin is the polymeric extracellular matrix protein responsible for the elasticity of lungs, skin, the bladder, the uterus, and large blood vessels. Elastin self-assembly involves a process of liquid-liquid phase separation called coacervation. Although the structure of elastin in the resulting matrix is unknown, the source of elastic recoil is known to be primarily entropic. Two different sources of entropy have been proposed in seemingly-contradictory models: rubber-like models attribute the elasticity of elastin to the entropy of the polypeptide chain in random-coil conformation, whereas ''liquid drop'' models ascribe the recoil of this highly-hydrophobic protein to the hydrophobic effect. Although its self-organization and mechanical properties have spurred interest in elastin as a model for useful biomimetic polymers, the molecular determinants of these properties are poorly understood.
We use atomistic molecular dynamics simulations in explicit solvent to examine the structural basis for the self-assembly and mechanical properties of elastin-like polypeptides in water. Massive sampling over simulation times totalling 0.3 millisecond indicates that the peptides remain highly disordered (though not random) even in the aggregated state, and provides an ensemble description of phase-separated peptide aggregates in which the polypeptide chains exhibit liquid-like properties approaching those of a polymer melt. In this phase-separated state, the peptides are largely solvated by one another, although the polypeptide backbone remains significantly hydrated. Crucially, both conformational entropy and hydrophobic burial drive the formation of this protein-rich phase, which may be called the liquid state of proteins. These findings support a unified model of self-assembled elastomeric proteins in which these two entropic forces play essential roles in both self-assembly and elastic recoil.

196-Symp
Decoding Molecular Plasticity Underlying Nucleocytoplasmic Transport: from Single Molecules to Large Assemblies
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Intrinsically disordered and phenylalanine-glycine rich nucleoporins (FG-Nups) form a selective permeability barrier inside the nuclear pore complex (NPC): Large molecules can only cross the central channel of the NPC when piggybacked by nuclear transport receptors (NTRs) that specifically interact with FG-Nups. These FG-Nups, however, display complex and non-random amino acid architecture and possess repeatedly occurring FG-motifs flanked by distinct amino acid stretches. How such heterogeneous sequence composition relates to function and how homotypic interactions between such disordered stretches, and transient heterotypic interactions with folded transport receptors could give rise to a transport mechanism is still unclear. We have now developed an integrated chemical biology-fluorescence approach to study the molecular plasticity of FG-Nups on the single-molecule level using multi-parameter fluorescence spectroscopy. Despite its heterogeneous primary sequence, the unstructured FG-domain of Nup153 displays a collapsed coil behavior across its entire amino acid sequence, due to favorable intrachain interactions. We show that those interactions at the dynamic disordered state can induce aggregation and lead to the formation of stable amyloid fibers that, at high protein concentrations, can further enlace to form macroscopic hydrogels with NPC like properties. Amyloid formation can also be inhibited by the presence of NTRs. Furthermore, we found that Nup153 retains its collapsed conformation even when involved in NTR•Nup complexes. Using fluorescence lifetime and polarized fluorescence fluctuation analysis with picosecond resolution, we were able to observe the formation of very flexible and dynamic complexes and detect novel binding modes underlying the nucleocytoplasmic transport process. Synergistic molecular dynamics simulations permit visualization of previously unknown steps and determinants during Nup•NTR interactions at atomic resolution. These results provide important insights on how nuclear transport can pursue specifically and very fast inside the nuclear pore complex.